

The University of Chicago

TRANSLOCATION OF CARBOHY-
DRATES IN THE CUTHBERT
RASPBERRY

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY

1938

By

CHARLES J. ENGARD

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CHARLES J. ENGARD

(WITH FOUR FIGURES)

Introduction

Many investigations have been conducted on the raspberry plant, especially in regard to the economically important aspects such as resistance to freezing, pruning, fertilizing, etc., but there has been little study relative to the forms and movements of the chief organic substances. DU SABLON (15) included the raspberry in his study of the reserve materials of trees. BRIERLEY (2, 3) studied senescence in the red raspberry cane, emphasizing cambial decline and phloem disintegration, and the responses to pruning. BRIERLEY and LANDON (4) presented evidence on the downward movement of carbohydrates in the fruiting canes. BENNETT (1) demonstrated that the movement of the leaf-curl virus of raspberries takes place in the phloem and in the direction of the major transport of food. Reviews of the literature in translocation have been published by CLEMENTS (8), CURTIS (14), and more recently by MASON and PHILLIS (22) and CRAFTS (13).

Material and methods

The Cuthbert raspberry is a hybrid derived from *Rubus idaeus* L. and *R. strigosus* Michx., the latter considered by HEDRICK (17) as a

¹ Contribution no. 57 from the Botany Department of the State College of Washington.

subspecies of *R. idaeus*. It produces tall erect canes which attain their full height and most of their diameter growth within the first growing season. Fruiting occurs in the second season on laterals developed from over-wintered buds. At the height of the harvest season disintegration of the phloem occurs at the base and in the apical region of the cane. BRIERLEY (2) thinks the former is associated with the senile condition of the cambium, and the latter caused by a disturbance in water conduction.

Because the various canes are relatively uniform in size, length, and diameter, the Cuthbert raspberry was chosen for experimental material. It was at first intended to include second year (fruiting) canes as well as first year ones, thus making a complete study up to and beyond fruiting; but during the winter previous to the beginning of this study all canes were winter killed, and none which could be used during the following growing season could be found in the locality.

The experimental plants were mosaic-free stock grown by the Department of Horticulture at the State College of Washington.² They were grown on a uniform Palouse silt loam soil with ample moisture for growth, and on a southwest slope. The rainfall of this region comes mostly during the winter and spring; after May gradual drying of the soil occurs up to the end of September, when there are occasional showers.

In this study double ringing was employed. By ringing at a point 15 cm. and again at a point 30 cm. above the ground, a segment of bark 15 cm. in length was isolated. The strips of bark removed were less than 1 cm. wide, completely girdling the stem. The lower ring prevents upward movement in the bark of certain substances from the roots and other normal canes growing therefrom, and the upper ring prevents downward movement of materials mobile in the bark into the isolated segment below.

On May 15, 1936, when the new shoots were about 60 cm. high, some were ringed, some tagged for controls, and others were taken into the laboratory and prepared for subsequent analysis. The plants harvested at the time of ringing on May 15 served as checks

² Thanks are due to the Department of Horticulture, State College of Washington, for the raspberry plants used throughout this investigation.

for the June series. Ringed canes and the controls tagged on May 15 were harvested on June 23, and another set of canes were ringed and others tagged for additional controls on this date. These were harvested on July 16 and constitute the July series. On July 16 canes were ringed, and others tagged for controls; these were harvested on August 9, as the August series. Control plants for any series (except May) were those which were tagged but not ringed in the immediately preceding month. Twenty-four canes were used in each treatment, twelve for nitrogen and twelve for carbohydrate analyses.

At each collection the canes were severed at the ground level. Each cane was cut into 15 cm. segments, measuring from the base, so that the sample contained the equivalent segment of twelve canes. The leaves of each segment were cut off at the junction of petiole and stem and were analyzed in aggregates of twelve equivalent segments. The stem samples were analyzed separately. The top segment (or portion thereof) and its young leaves were analyzed as one sample. For these various samples the following symbols have been adopted: (1) Each 15 cm. segment of stem, numbering from the base to the top, is designated as 1st, 2st, 3st, etc. Thus in the double ringed canes, segment 1st is that below the lower ring; segment 2st is that between the rings; and segment 3st is that above the upper ring. (2) The several leaves diverging from each segment are symbolized as 3L, 4L, 5L, and 6L, these being from segments 3st, 4st, 5st, and 6st respectively. The leaves usually drop from the first 30 cm. of the raspberry stem, so that in these experiments the leaves were cut from the first two segments at each treatment. (3) The uppermost segment (or portion thereof) with leaves too small to constitute a separate sample, is designated as tops.

The segmentation was done as rapidly as possible, and all samples were immediately weighed and autoclaved at 15 lb. pressure for 15 minutes. After drying in a ventilated oven heated to 60° C., the material for carbohydrate determinations was ground to 40-mesh fineness in a power mill.

MOISTURE.—The moisture content of the carbohydrate samples was determined by drying them for a week in an oven at 60° C. and then weighing.

CARBOHYDRATES.—The procedure was essentially that described by CLEMENTS (6), with the exception that the term “acid hydrolyzable carbohydrates” has been substituted for “hemicellulose.” This group was taken out in two fractions (11), one after three hours of hydrolysis on the steam bath and the second after twelve hours. The first group, designated as “acid hydrolyzable A,” is presumably made up of polysaccharides of high molecular weight which are held more or less loosely to the cell wall or within the protoplast. The second group, “acid hydrolyzable B,” is made up of polysaccharides which are more stable and which are possibly part of the secondary thickening of the cell wall.

TABLE 1
DISTRIBUTION OF CARBOHYDRATES IN NORMAL PLANTS
MAY 15 (BEGINNING CONTROLS)

CANE SEGMENT	TOTAL CARBOHYDRATE		REDUCING SUGARS		SUCROSE		STARCH		TOTAL ACID HYDROLYZABLE	
	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)
1st.....	5.80	27.70	1.40	06.0	0.06	0.24	0.18	0.77	4.16	17.70
2st.....	4.98	31.13	1.67	10.4	0.07	0.46	0.23	1.44	2.91	18.18
3st.....	2.72	33.18	1.20	14.7	0.07	0.83	0.18	2.20	1.27	15.49
Tops.....	2.53	33.72	1.04	13.9	0.05	0.71	0.18	2.40	1.26	16.80
3L.....	12.20	36.40	4.94	14.1	0.05	0.15	0.07	0.21	5.71	17.05

EXPRESSION OF RESULTS.—The basis upon which to calculate and report analytical results is a prevalent controversy. In the light of considerations to be reported elsewhere (16), the following procedure is used in expressing results: (1) When it is especially desirable, absolute changes in a segment are expressed as the weight difference in grams per sample (equivalent segments of twelve canes = a sample). (2) Percentage concentrations are used in most cases, such as those dealing with gradients. The substances of the control plants are expressed as percentages of their residual dry weight. The substances of the treated plants are expressed as percentages of the residual dry weight of the corresponding controls.

Investigation

SEASONAL DISTRIBUTION OF CARBOHYDRATES IN NORMAL PLANTS

TOTAL CARBOHYDRATES.—The percentage of total carbohydrates, which includes the material hydrolyzable in 2.5 per cent acid in fifteen hours, is fairly high in the stem and leaves of the plants harvested in May (table 1). It drops during June, the period of most

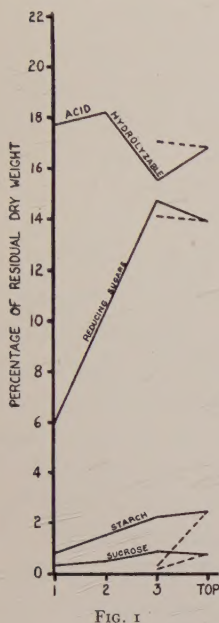


FIG. 1

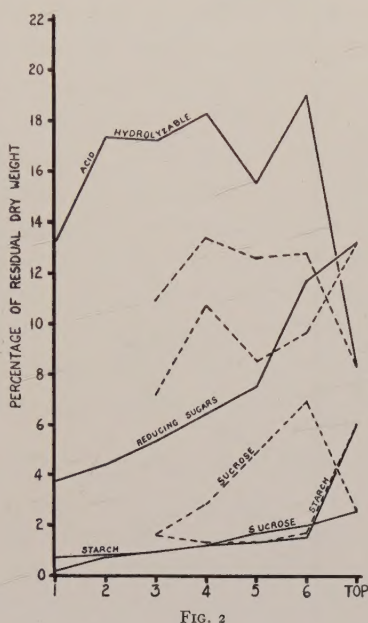


FIG. 2

FIGS. 1, 2.—Fig. 1 (left), distribution of carbohydrates in normal cane segments, May 15. Dotted lines represent leaf samples corresponding to stem segments. Cane segments, numbering from base of stem to top, are indicated along abscissa. Fig. 2 (right), distribution of carbohydrates in normal cane segments, June 23.

rapid growth, and attains a maximum in August. The drop during the period of rapid growth corresponds to that observed by DU SABLON (15) in the raspberry. He attributed the early spring high to a migration of carbohydrates from the roots into the stems. He obtained a maximum of total carbohydrates, as percentages of the dry weight, in October. The concentrations of total carbohydrates are slightly lower in the lower segments than in the upper in the May

and June collections. This ascending order practically disappears during July and August. All fractions of the total carbohydrates for the normal plants are plotted in figures 1 to 4.

ACID HYDROLYZABLE CARBOHYDRATES.—This group of substances, the largest fraction of the total carbohydrates, made up of heterogeneous cellulosic compounds—polysaccharides of high molecular

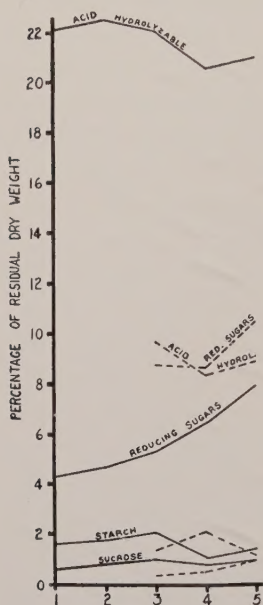


FIG. 3

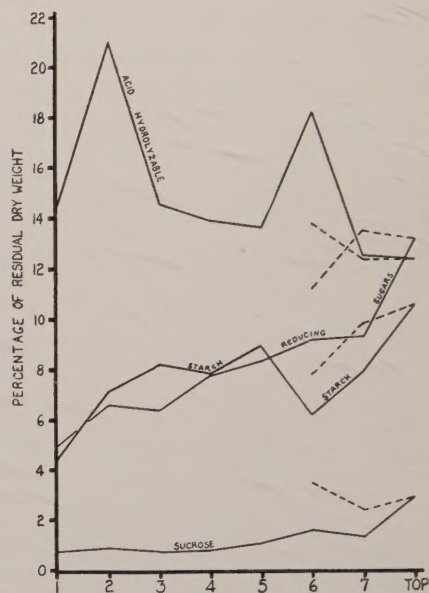


FIG. 4

FIGS. 3, 4.—Fig. 3 (left), distribution of carbohydrates in normal cane segments, July 16. Fig. 4 (right), same, August 9.

weight and their derivatives—have varying degrees of stability upon hydrolysis. For this reason, hydrolysis for three hours and then for an additional twelve hours yielded very erratic results. For example, the concentration of the former or A fraction varied from 1 per cent in one segment to 10 per cent in the neighboring segment, whereas the second or B fraction would be the reverse. The total of the two fractions in any one segment, however, was comparable with that of the other segments. The idiosyncrasies of the less stable “hemicelluloses” disappeared upon continued hydrolysis, although some in-

consistency still remained. For this reason the individual A and B fractions of the acid hydrolyzable substances were not graphed nor tabulated. Separate hydrolysis, however, has the advantage of largely preventing the destruction of that portion of the hydrolysate which is produced early in the boiling process.

With the exception of the May series, the tops are lowest in concentration of these substances. The second segment is always considerably higher in concentration than the first, and with the exception of the June series the concentration in the second segment is the highest of all parts of the plant. This may be owing to its location just below the foliage region, for, as BRIERLEY (2) has shown for fruiting canes, cambial activity is greatest in the region below a vigorous lateral or foliage, and is usually absent in the base of the cane. In 90 per cent of the canes measured by BRIERLEY (2) he found no measurable increase in diameter at the base of old canes. There may be some relationship between the erratic activity of the raspberry cambium in various regions of the stem and the erratic results obtained for the acid hydrolyzable material along the stem, but the results are probably caused by the variable responses of the substances to hydrolysis.

In all but the May series the amount of acid hydrolyzable material is lower in the leaves than in the stem segments. The increase of the acid hydrolyzable fraction in the tops of the May series is accompanied by a drop in the reducing sugars. The graph of this drop (fig. 1) from 3st to tops, and the leaves of 3st to tops, is nearly a mirror image of the graph of the acid hydrolyzable substances in the same region. The amounts of reducing sugars in this series are higher than in any other collection, but starch and sucrose are lower. The highest concentration of sugars is nearly 700 per cent higher than that of starch. There seems to be a close relationship in the May series between the high concentrations of reducing sugars and the acid hydrolyzable carbohydrates. This indicates that not only are the "hemicelluloses" a form of food reserve, but they may be an even more immediate storage form than starch. Thus it is concluded with CLEMENTS (6) that, "the vacuolar sugars may be so concentrated as to favor the formation of some reserve which is entirely removed from the solution," and that, "under favorable conditions

for rapid carbohydrate formation they [the hemicelluloses] seem to act as temporary reserves in the leaves."

REDUCING SUGARS.—The highest concentrations of reducing sugars are in the May series, drop rapidly in the June series, from whence they increase only slightly in July and August. The basal segment is always lowest in reducing sugars, with concentrations increasing toward the top. The leaves diverging from the subterminal segment have less sugars than that segment, except in the August series. Lower leaves are always higher in concentration of sugars than their corresponding segment. The large amount of reducing sugars in all parts of the plant seems to indicate that it is an important temporary storage form.

STARCH.—Starch is never abundant, and does not rise above 6 per cent except in the August series, where it essentially parallels the reducing sugar curve. Apparently it is not an important reserve in the first year canes of raspberry. The starch content of leaves is lower than that of the corresponding stem segments in May, is equivalent in June, slightly above in July, and definitely higher in August. The gradient is not steep and the basal segment is lowest in starch content.

SUCROSE.—Sucrose is present at all times in all parts of the plant, but its concentration is low, rising above 2 per cent only in June and in August. It becomes highly concentrated in the leaves of the June series, reaching a maximum in the uppermost and grading downward to the lowermost leaves.

The very low concentration of this sugar indicates that it is not an important one, either for direct use as an energy source or for temporary storage.³ Its presence throughout the plant suggests that it has a definite role in metabolism, probably of translocation. That it does not result as a condensation product when reducing sugars attain a certain maximum concentration, such as CLEMENTS (6) found in his work on the sunflower, soy bean, and potato, is indicated by an entire lack of relationship between the varying concentrations of reducing sugars and the little-fluctuating amounts of sucrose. There seems to be no relationship with moisture content, as LEONARD (18)

³ Microchemical data to be presented in a later paper indicate that sucrose is limited in its distribution, essentially to phloem, making it inconceivable that sucrose functions as an important source of energy.

found in the sunflower. The very small amounts of sucrose in the stem indicate that, if it is a translocatory form, it is confined to a very small part of the total mass of stem, presumably the phloem. The actual concentrations in this tissue are thus greatly reduced by the total mass of the stem. The slight gradient which appears in figures 1 to 4 is insignificant and is probably nonexistent. It is the result of dividing the grams of sucrose by residual dry weights which are somewhat greater in the lower than in the upper portions of the plant.

EFFECT OF RINGING

The effects of double ringing on the sugars in the treated plants are compared with the untreated plants in tables 2 to 4. The usual accumulation of carbohydrates is found in the parts above the rings owing to the prevention of movements into regions below the ring. According to MASON and MASKELL (20), ringing is noticeably effective in the interruption of the downward movement of carbohydrates within two hours. In all series there is a great increase of total carbohydrates above the rings over the normal concentrations. Accompanying this increase is an even greater decrease of total carbohydrates between and below the rings.

Increases or decreases in the ringed canes, expressed in whole numbers as percentages of the normal concentrations, are given for the segments below the rings (1st), between the rings (2st), and just above the rings (3st) in table 5. It is evident that all carbohydrate substances, except starch in the June series, make large percentage increases in the segment above the upper ring. The largest increase was in the amount of sucrose. The percentage increase of reducing sugars was only half that made by sucrose.

All fractions decrease greatly between the rings. Sucrose again was affected most, there being a 75 per cent reduction in June, and a 100 per cent reduction in July and August. The reducing sugars showed not so great an apparent decrease, but much of that present at the time of analysis was undoubtedly derived from starch and acid hydrolyzable carbohydrates, as well as from the residual polysaccharides (table 7). The data in tables 5 and 7 indicate that carbohydrates other than reducing sugars were 43 to 100 per cent lower than normal.

TABLE 2
SEASONAL CHANGES IN TOTAL CARBOHYDRATES AND
REDUCING SUGARS IN NORMAL AND RINGED PLANTS

CANE SEGMENT	JUNE 23				JULY 16				AUGUST 9			
	CONTROLS		RINGED		CONTROLS		RINGED		CONTROLS		RINGED	
	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)
TOTAL CARBOHYDRATES												
1st.....	4.00	17.75	1.67	7.44	6.60	28.41	3.82	16.47	8.20	35.68	5.10	22.15
2st.....	3.70	22.98	1.13	6.99	5.10	29.48	2.81	16.23	6.40	35.55	2.28	12.67
3st.....	3.30	23.73	5.12	36.85	4.40	30.55	5.54	38.40	4.80	29.45	7.73	47.40
4st.....	3.10	28.43	4.27	39.25	2.80	28.90	4.52	46.70	3.90	30.00	7.02	54.00
5st.....	2.10	26.25	3.03	37.85	2.50	31.28	4.14	51.75	3.10	31.60	4.33	44.30
6st.....	1.40	34.15	1.93	47.10					2.70	34.60	4.01	51.40
7st.....									1.90	30.15	2.79	44.30
Top.....	3.80	29.45	3.55	27.50					5.90	38.80	6.95	45.70
7L.....									6.10	37.45	13.85	85.00
6L.....	5.20	30.75	5.45	32.20					6.30	36.22	15.44	88.80
5L.....	5.60	27.45	5.87	28.80	5.80	29.00	6.10	30.50				
4L.....	5.40	28.28	6.10	31.95	4.20	23.60	5.70	32.20				
3L.....	3.50	21.07	5.17	31.15	4.60	22.45						
REDUCING SUGARS												
1st.....	0.85	3.78	0.37	1.64	0.99	4.27	1.28	5.52	1.14	4.96	0.93	4.04
2st.....	0.71	4.40	0.23	1.45	0.82	4.64	0.61	3.53	1.18	6.55	0.49	2.72
3st.....	0.74	5.35	1.52	10.90	0.76	5.28	1.24	8.61	1.03	6.30	2.75	16.87
4st.....	0.70	6.43	1.24	11.40	0.62	6.39	0.95	9.80	1.01	7.77	2.06	15.85
5st.....	0.60	7.50	1.14	14.20	0.63	7.88	1.05	13.14	0.81	8.27	1.50	15.32
6st.....	0.47	11.75	0.76	18.45					0.71	9.10	1.09	14.00
7st.....									0.58	9.20	1.06	16.82
Top.....	1.70	13.19	1.64	12.70					1.98	13.02	3.02	19.88
7L.....									2.17	13.30	6.00	36.80
6L.....	1.63	9.64	2.09	12.35					1.94	11.15	6.29	36.15
5L.....	1.75	8.58	2.24	10.95	2.08	10.40	2.25	11.30				
4L.....	2.06	10.78	2.49	13.04	1.52	8.54	1.84	10.33				
3L.....	1.18	7.11	2.34	14.53	1.80	8.77						

TABLE 3

SEASONAL CHANGES IN SUCROSE AND STARCH IN NORMAL AND RINGED PLANTS

CANE SEG- MENT	JUNE 23				JULY 16				AUGUST 9			
	CONTROLS		RINGED		CONTROLS		RINGED		CONTROLS		RINGED	
	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)	Gm.	RESID- UAL DRY WEIGHT (%)	Gm.	CON- TROL RESID- UAL DRY WEIGHT (%)
SUCROSE												
1st....	0.03	0.133	0.092	0.408	0.13	0.561	0.354	1.525	0.16	0.695	0.378	1.641
2st....	0.11	0.684	0.027	0.167	0.13	0.752	0.000	0.000	0.15	0.834	0.000	0.000
3st....	0.12	0.863	0.454	3.270	0.14	0.972	0.312	2.165	0.11	0.675	0.422	2.530
4st....	0.13	1.192	0.326	2.990	0.07	0.721	0.311	3.200	0.10	0.770	0.883	6.790
5st....	0.13	1.625	0.262	3.270	0.07	0.875	0.291	3.540	0.10	1.020	0.336	3.430
6st....	0.08	1.950	0.213	5.180					0.12	1.540	0.683	8.750
7st....									0.08	1.260	0.149	2.360
Top...	0.32	2.480	0.694	5.380					0.43	2.830	1.826	12.000
7L....									0.39	2.390	1.755	10.875
6L....	1.17	6.930	1.119	6.620					0.60	3.430	2.810	16.125
5L....	1.00	4.910	1.850	9.070	1.75	0.875	0.693	3.465				
4L....	0.54	2.830	1.498	7.850	0.81	0.456	0.713	4.000				
3L....	0.27	1.623	0.891	5.375	0.59	0.288						
STARCH												
1st....	0.15	0.667	0.000	0.000	0.37	1.595	0.112	0.482	1.00	4.350	1.111	4.840
2st....	0.12	0.745	0.025	0.155	0.30	1.735	0.000	0.000	1.28	7.120	0.160	0.890
3st....	0.12	0.854	0.064	0.460	0.29	2.015	0.645	4.482	1.33	8.160	1.870	11.475
4st....	0.13	1.191	0.056	0.511	0.10	1.048	0.608	6.270	1.01	7.770	1.825	14.050
5st....	0.10	1.250	0.062	0.769	0.11	1.376	0.633	7.910	0.86	8.780	1.062	10.850
6st....	0.06	1.402	0.028	0.685					0.48	6.160	1.044	13.400
7st....									0.50	7.940	0.853	13.550
Top...	0.78	6.050	0.166	1.289					1.59	10.460	0.748	4.360
7L....									1.59	9.750	4.360	26.740
6L....	0.28	1.657	0.132	0.778					1.34	7.710	2.355	13.520
5L....	0.27	1.323	0.119	0.582	0.22	1.100	1.300	6.500				
4L....	0.25	1.310	0.105	0.549	0.36	2.020	0.989	5.560				
3L....	0.26	1.508	0.044	0.265	0.28	1.366						

All fractions except sucrose and the reducing sugars in the July series showed a decrease in the basal segment. In all cases the decrease was less in July and August, owing to the duration of the treatment for only three weeks instead of five and a half weeks as in the June series.

TABLE 4
SEASONAL CHANGES IN ACID HYDROLYZABLE SUBSTANCES
IN NORMAL AND RINGED PLANTS

CANE SEG- MENT	JUNE 23				JULY 16				AUGUST 9			
	CONTROLS		RINGED		CONTROLS		RINGED		CONTROLS		RINGED	
	GM.	RESID- UAL DRY	GM.	CON- TROL RESID- UAL DRY	GM.	RESID- UAL DRY	GM.	CON- TROL RESID- UAL DRY	GM.	RESID- UAL DRY	GM.	CON- TROL RESID- UAL DRY
		WEIGHT (%)		WEIGHT (%)		WEIGHT (%)		WEIGHT (%)		WEIGHT (%)		WEIGHT (%)
1st....	2.97	13.20	1.214	5.355	5.14	22.15	2.077	8.953	3.32	14.43	2.605	11.325
2st....	2.78	17.28	0.840	5.212	3.89	22.48	2.204	12.740	3.79	21.05	1.634	9.070
3st....	2.39	17.19	3.089	22.230	3.18	22.08	3.343	23.300	2.36	14.47	2.683	16.424
4st....	2.00	18.34	2.152	19.750	1.99	20.52	2.640	27.215	1.80	13.85	2.262	17.390
5st....	1.24	15.50	1.573	19.660	1.68	21.00	2.177	27.250	1.33	13.57	1.425	14.540
6st....	0.78	19.01	0.945	23.020					1.41	18.10	1.206	15.449
7st....									0.78	12.39	0.725	11.500
Top...	1.07	8.29	1.047	8.114					1.86	12.24	1.351	8.888
7L....									2.00	12.25	1.714	10.501
6L....	2.15	12.73	2.093	12.385					2.41	13.86	3.979	22.880
5L....	2.57	12.61	1.603	8.308	1.78	8.90	1.830	9.159				
4L....	2.55	13.35	1.995	10.437	1.48	8.32	2.152	12.083				
3L....	1.82	10.96	1.894	11.410	1.98	9.67						

Sucrose made remarkable gains in the basal segment, in some instances equaling and even surpassing the increase in the segment above the rings. The large increases above the ring, 100 per cent greater than the increases of reducing sugars, indicate very strongly that sucrose serves only as a translocatory form moving downward in the stem. However, increases of the same magnitude below the rings are equally strong evidence that sucrose is the main translocatory form moving upward from the roots into the stem. Thus ringing brings about a bidirectional movement in the canes, which is con-

TABLE 5
DECREASES (−) OR INCREASES (+) AS PERCENTAGE
OF NORMAL IN FIRST THREE SEGMENTS
OF RINGED STEMS

FRACTION	CANE SEGMENT	JUNE 23 (%)	JULY 16 (%)	AUGUST 9 (%)
Total carbohydrates	{1st.....	− 58	− 42	− 38
	{2st.....	− 70	− 45	− 81
	{3st.....	+ 57	+ 25	+ 61
Total acid hydrolyzable	{1st.....	− 59	− 59	− 21
	{2st.....	− 70	− 43	− 57
	{3st.....	+ 29	+ 5	+ 13
Reducing sugars	{1st.....	− 53	+ 28	− 25
	{2st.....	− 66	− 24	− 59
	{3st.....	+102	+ 62	+116
Sucrose	{1st.....	+211	+173	+134
	{2st.....	− 75	−100	−100
	{3st.....	+283	+124	+272
Starch	{1st.....	−100	− 70	+ 11
	{2st.....	− 79	−100	− 86
	{3st.....	− 46	+122	+ 41

TABLE 6
GRADIENTS IN REDUCING SUGARS AND SUCROSE
IN NORMAL AND RINGED CANES

	JUNE 23					AUGUST 9					
	TOTAL (GM.)	GM./CM.	6L TO 6ST (GM.)	5L TO 5ST (GM.)	6L TO 3L (GM.)	TOTAL (GM.)	GM./CM.	7L TO 7ST (GM.)			
	1ST TO TOPS					1ST TO TOPS					
Normal											
Reducing sugars....	+9.40	+0.100	-2.20	+1.1	+2.5	+8.00	+0.07	+4.1			
Sucrose.....	+1.62	+0.018	+4.98	+3.3	+4.3	+2.14	+0.02	+1.1			
	3ST TO TOPS					3ST TO TOPS					
Ringed											
Reducing sugars....	+1.8	+0.030	-6.1	-3.0	-2.1	+3.0	+0.04	+20.0			
Sucrose.....	+2.1	+0.035	+1.4	+5.8	+1.2	+10.5	+0.14	+14.5			

TABLE 7
CHANGES IN RESIDUAL DRY WEIGHT IN FIRST FOUR SEGMENTS OF NORMAL AND RINGED CANES
OF JUNE AND AUGUST COLLECTIONS

CANE SEGMENT	MAY 15			JUNE 23						JULY 16			AUGUST 9					
	NORMAL			NORMAL			RINGED			NORMAL			NORMAL			RINGED		
	RESID- UAL	TOTAL CAR- BOHY- DRATES (GM.)	WEIGHT (GM.)	RESID- UAL	TOTAL CAR- BOHY- DRATES (GM.)	WEIGHT (GM.)	RESID- UAL	TOTAL CAR- BOHY- DRATES (GM.)	WEIGHT (GM.)	RESID- UAL	TOTAL CAR- BOHY- DRATES (GM.)	WEIGHT (GM.)	RESID- UAL	TOTAL CAR- BOHY- DRATES (GM.)	WEIGHT (GM.)	RESID- UAL	TOTAL CAR- BOHY- DRATES (GM.)	WEIGHT (GM.)
	DRY	BOHY- DRATES (GM.)	WEIGHT (GM.)	DRY	BOHY- DRATES (GM.)	WEIGHT (GM.)	DRY	BOHY- DRATES (GM.)	WEIGHT (GM.)	DRY	BOHY- DRATES (GM.)	WEIGHT (GM.)	DRY	BOHY- DRATES (GM.)	WEIGHT (GM.)	DRY	BOHY- DRATES (GM.)	WEIGHT (GM.)
1st.....	23.5	5.8	22.5	4.0	8.3	1.7	-63.0	-57.5	23.2	6.6	23.0	8.2	19.4	5.1	15.6	-37.8	-37.8	-37.8
2nd.....	16.0	5.0	16.1	3.7	8.9	1.1	-44.7	-70.4	17.3	5.1	18.0	6.4	13.4	2.3	-25.6	-64.0	-64.0	-64.0
3rd.....	8.2	2.7	13.9	3.3	16.2	5.1	+23.7	+54.5	14.4	4.4	16.3	4.8	20.2	7.7	+23.9	+60.4	+60.4	+60.4
4th.....			10.9	3.1	11.5	4.3	+5.5	+38.8	9.7	2.8	13.0	3.9	16.8	7.0	+22.6	+79.5	+79.5	+79.5

* Increase (+) or decrease (-) as percentage of normal.

firmatory of evidence based on virus activity by BENNETT (1). In addition to the accumulation of sucrose above the rings, an accumulation of reducing sugars also occurs, indicating that some of the latter move downward in the bark.

CARBOHYDRATE GRADIENTS

All carbohydrate gradients in the stem are positive; that is, they descend from a high concentration in the top to a low concentration in the base.⁴

The gradients are positive for insoluble as well as for soluble carbohydrates, and therefore do not give evidence of the direction or the form of movement. Girdling may increase or decrease the gradients but they always remain positive.

As was found by MASON and MASKELL (20, 21), sugars move downward with a marked positive gradient (table 6). Unlike their results, however, is the fact that ringing does not cause the gradient to disappear (table 6). It reduces the positive gradient of reducing sugars per stem-centimeter and steepens the gradient of sucrose per stem-centimeter (table 6). Ringing also reverses the positive gradient of reducing sugars from leaves to stem in the fourth and fifth segment of the June series. The sucrose gradient from leaves to stem segment is reduced but remains positive.

The positive gradients of acid hydrolyzable carbohydrates and of starch are stable; that is, they are gradients produced by storage, temporary or permanent, of the two groups of substances. These are static gradients brought about by accumulations within living cells or cells associated with living cells. The proportion of living to "dead" cells is much greater in the tops, upper leaves, and the neighboring subterminal stem segment than in the lower portions. The amount of meristematic activity becomes less in proportion to the amount of secondary xylem, cork, and fibers produced toward the basal segment. An important contributory factor to the positive gradients of the static carbohydrates is the increase from the top to the base of the stem in residual dry weight by which the percentages are determined.

⁴ MASON and MASKELL (20) defined gradients as positive—high concentrations in the upper and low concentration in the lower portions, and negative—the reverse.

The positive gradient of reducing sugars is for the most part a static one of temporary storage sugars accumulated within living cells and associated with metabolism. Sugars may accumulate against a gradient of reducing sugars. MASKELL and MASON (19) demonstrated that movement of sucrose took place with a positive gradient into the cotton boll, in which the concentration of reducing sugar was much higher than in the stem. Reducing sugars thus accumulated as a storage sugar in the boll. Although in the raspberry experiments bark and wood were not analyzed separately, microchemical data show that much of the reducing sugars contributing to the static gradients are stored in the xylem, pith, and rays.

Part of the positive gradient of reducing sugars is undoubtedly translocatory, as is evidenced by the accumulation of these sugars above the ring (table 5), and the consequent decrease of the gradient (table 6). This is also evidence that the bark, and presumably the phloem, is the region in which the translocatory reducing sugars are moving. The data of tables 5 and 6 are also indicative of the downward movement of reducing sugar.

It remains to explain why, despite a 200 per cent increase in sucrose above the rings, the insignificant sucrose gradient actually increases considerably. It would normally decrease, or if nonexistent would become negative, if sucrose were moving downward in the bark. The evidence for downward movement of sucrose shown by one datum (increase above the rings) seems paradoxical in view of the evidence for upward movement by the other datum (increase below the rings). The canes used in these investigations are annual canes, but they are growing from perennial, sugar-gorged roots to which many other canes supply carbohydrates. If translocation of sugar is considered as polar from a source (the leaves) to a "sink" (the roots), downward movement will proceed along a positive longitudinal gradient (20, 21). The situation is different with the raspberry, however, for there is in the young first year canes apparently no well defined polarized movement. DU SABLON (15) explained the April maximum of carbohydrates in the first year canes of the raspberry as brought about by a migration of carbohydrates from the roots. Thus it is apparent that, in the very early stage of development of the vegetative canes, the usual downward movement

of mobile sugars common to most annuals and perennials is reversed; that is, there is established a temporary root-to-shoot gradient, even though the shoot is highly photosynthetic. During the ensuing growth period the early spring root-to-shoot gradient gradually disappears, owing to the increased production of sugars by the rapidly enlarging foliage region of the cane. Reducing sugars are produced in far greater abundance than sucrose, and the leaves-to-root gradient is soon established. Sucrose is formed in small amounts, and seems to be confined to the bark. The production of an abundance of sucrose in the upper regions of the cane is much slower than the production of an abundance of reducing sugars. During the first season, therefore, the downward movement of sucrose seems to be counterbalanced by an upward movement, seemingly in the same channel, and the net result is a static condition with no apparent gradient. As pointed out previously in this paper, the slight gradients of sucrose, as shown in figures 1 to 4 and in table 6, are thought not to be significant because the gradient exhibited is produced by dividing by a higher residual dry weight in the lower segments than in the upper segments. The gradient apparently does not exist. The effect of a ring on this situation is to reverse the influence of the opposing source of sucrose on the other gradient. The result is an accumulation of sucrose in the segment above and below the isolated segment, and a steepening of the positive gradient above the rings. No data were obtained on the gradient below the rings, but on the basis of the preceding explanation a negative gradient would appear. No analyses were made on the roots.

USABILITY OF RESIDUAL POLYSACCHARIDES

Since the introduction by SCHULZE (29) of the term "hemicellulose" to denote a cell wall substance similar in nature to cellulose, ever increasing data point to this group of substances as being of definite physiological significance. DU SABLON (15) demonstrated an accumulation of hemicellulose in the fall and a subsequent utilization in the following spring. SCHELLENBERG (28) showed that hemicellulose is utilized as a reserve food by trees, and CASTORO (5) thought that the hemicellulose which he found in the seeds of *Ruscus aculeatus*

functioned as a reserve food. TOTTINGHAM *et al.* (31) assigned to them the function of serving as reserve carbohydrates in apple trees, and ROSA (27) assigned to them a distinct function in the winter hardening process of plants. NIGHTINGALE (25) presented data which indicated that hemicellulose is utilized by the plant as a source of sugar. MURNEEK (24) discussed the significance of hemicellulose as a reserve carbohydrate in apple spurs. CLEMENTS (6) presented convincing evidence that in the sunflower, soy bean, and potato the hemicelluloses are significantly functional in several roles: (1) they serve as temporary reserves under favorable conditions for carbohydrate synthesis; (2) their accumulation and maintenance add to the drought resistance of the plant; (3) they play a morphic role by contributing to the strength of tissues as cell wall thickenings; and (4) they serve as energy reserves in stem and roots. Many investigations have since been reported in which the role of hemicelluloses in drought resistance has been confirmed. Nevertheless LOOMIS, in a report assembled for the committee on chemical methods of the American Society of Plant Physiologists (12), states with regard to the hemicelluloses, "The physiological significance of these materials has not yet been established." Recently CLEMENTS (9), on the basis of evidence in the soy bean, suggested a mechanism of drought resistance in which the gel-forming properties of the hemicelluloses, which not only line the inner walls of the cells but extend into the protoplasm as well, play an important role.

WINKLER and WILLIAMS (32) have recently submitted data on the hemicellulose content of sections of grape stems which they think refute the contentions of the supporters of hemicellulose as a food reserve. They state, in regard to the work of MURNEEK (23), LEONARD (18), and CLEMENTS (6), that "the observed variations of the hemicellulose content were irregular, and furthermore, are not correlated with the vegetative condition of the plant. Their variations certainly do not point to hemicellulose as a reserve." However, these workers did find an unmistakable correlation of hemicellulose fluctuations with the vegetative condition of the plant (6, 9, 10). WINKLER and WILLIAMS found no significant variations in hemicellulose content of grape stems. They showed no data for the other carbohydrate fractions.

In all the work just mentioned the hemicelluloses were determined following acid hydrolysis of not more than six hours' duration. In this investigation the term acid hydrolyzable carbohydrates has been substituted for hemicellulose; furthermore, hydrolysis was carried out for fifteen hours. The quantities of substances extracted in this manner showed large variations in different parts of the plant, attesting their possible role as temporary food reserves.

The hemicelluloses have thus been established as playing a significant physiological role in metabolism. As such they are considered the most stable of the important carbohydrates which are actively associated with metabolism. Their function in metabolism is variable among plants, and depends to a great extent upon the kinds and amounts of other more readily utilizable carbohydrates. Their role may be a minor one in the normal life of some plants under good conditions for growth (32), but a change in conditions of growth will usually find its effect in the hemicelluloses. Soy beans react more favorably to conditions of drought than do potatoes and sunflowers, owing primarily to a greater abundance of hemicelluloses (9, 10).

Because they are a heterogeneous group of polysaccharides and their derivatives, it is extremely difficult accurately to define their chemical composition. It is likely that this group includes much of the "intussusception" material of the cell walls. The less dehydrated material is hydrolyzed first and in greatest amount. This is the material which is most available as hemicellulose reserve, and it is commonly obtained within a three hour period of hydrolysis. Hydrolysis for a fifteen hour period should yield most of the hemicellulose available for utilization in metabolism.

The total carbohydrates, which in this work included the substances hydrolyzable in 2.5 per cent acid in fifteen hours, were subtracted from the dry weight of the sample, and the residual dry weight was obtained as suggested by MASON and MASKELL (20). The residual dry weight consists of minerals (about 3-4 per cent of the dry weight, 7), fats, total nitrogen, and the stable cell wall material, including cellulose, which is not hydrolyzed in fifteen hours. The total organic nitrogen makes up 1-3 per cent of the residual dry weight, and fluctuates within this range. These substances—fats, minerals, and total organic nitrogen—do not at most account for

more than 5 per cent of the fluctuations in residual dry weight. In the ringed canes the latter deviates as much as 63 per cent of the normal (table 7). The great fluctuations in residual dry weight are therefore owing to fluctuations in residual polysaccharides.

The distributions, in grams per sample, of the residual substances in the first four segments of normal and ringed canes of the June and August series are given in table 7. Included in the table for comparison with the distributions of the June and August series are the figures for the May series. The deviations from the normal of the residual substances in the ringed canes are given in percentages. In the normal canes the residual substances, like the carbohydrates, are distributed in decreasing weights from the base toward the top. The weight gradient in grams is therefore opposite (negative) to the concentration gradient. In the first two segments the residual dry weight of the normal canes remained fairly constant throughout the season.

In the ringed canes the fluctuations of residual substances are in the same direction as those of the total carbohydrates. Where there is a loss of carbohydrates (see May and July figures, table 7), there is a loss of residual substances; and where there is an accumulation of carbohydrates there is a large increase in residual substances. An inversion of the residual polysaccharides takes place as the amount of carbohydrates is depleted by respiration. Above the rings the accumulation of total carbohydrates is faster than the condensation to the residual polysaccharides, owing to the influx of soluble forms from the foliage region. The evident responses of the residual polysaccharides to fluctuations in the total carbohydrates are definite indications of the equilibrium between these two groups. If a sufficiently large shift is produced by depletion or accumulation of the soluble forms, this shift will ultimately produce its effect in the residual polysaccharides. There are no data available which indicate how quickly the adjustment occurs, but the results presented here leave little doubt that it does take place.

COORDINATION OF RESULTS

In the normal distribution of the soluble carbohydrates the reducing sugars are far more abundant than sucrose. This abundance is

superseded only by the acid hydrolyzable substances whose activity seems to be closely associated with the reducing sugars. Their persistent abundance, coupled with the positive gradient of reducing sugars, is associated with the gradient of living cells. This leaves no doubt that the reducing sugars are the major immediate source of metabolic energy, as well as a temporary storage form. Removal of a ring of bark causes an accumulation of the reducing sugars above it, and in so doing decreases the gradient in the stem. These results indicate that a portion of the reducing sugars is functional in translocation in the bark. The positive gradient of reducing sugars in the normal canes is the sum of two components: (1) a static positive gradient of temporary storage sugars associated with active protoplasm; and (2) a dynamic positive gradient of reducing sugars which are translocatory.

The concentration of sucrose is very low at all times of the season. However, it is by far the most responsive to ringing of all forms, and this sensitivity is evidence of its role in the metabolism of the plant. Sucrose apparently is not produced in the raspberry by a fructose-glucose concentration maximum. Its gradient, if it has one, is so slight that it is well within the error produced by variations of the residual dry weight upon which the concentrations are expressed. It is thought that no gradient exists. Ringing causes a 200 per cent (of the normal) accumulation above and below the rings, and a 100 per cent decrease between the rings. The slight positive gradient which might exist is made more positive; if the gradient does not normally exist, a positive one appears. These data are interpreted to mean that:

1. Sucrose serves essentially as a translocatory form.
2. It moves in the bark, probably in the phloem.
3. There is an upward movement of sucrose from the perennial roots into the young shoot. It is probable that some of this sucrose is supplied by normal canes growing from the same roots.
4. As the developing cane enlarges and produces an increasingly greater photosynthetic area, sucrose is formed in the upper region of the cane and tends to move downward with its own gradient.
5. During the growth of the first year canes there is a period of adjustment of the two sources of sucrose. The gradient of one is

compensated by that of the other, and no gradient appears. It is likely that in the second year a definite positive gradient of sucrose appears, owing to the preponderance of the sugars produced by a large photosynthetic cane.

6. Ringing removes the influence of one source of sucrose upon the other, and an accumulation occurs above and below the isolated segment. A gradient, which before was compensated by one issuing from an opposing source, is made to appear.

It is significant that sucrose entirely disappears from the segment between the rings, whereas the reducing sugars are always present, although low in quantity. Unlike the reducing sugars, sucrose does not appear upon hydrolysis of the "hemicelluloses"; therefore sucrose is not a product of hydrolysis. Perhaps it is formed, in a manner similar to that found in the cotton plant by PHILLIS and MASON (26), within the sheath cells or companion cells of the phloem, and appears essentially in the phloem. In the raspberry it seems to have no function other than one of translocation.

Starch plays an insignificant role in the metabolism of the raspberry. BRIERLEY and LANDON (4) were unable to demonstrate microchemically the presence of starch, even in the fruiting canes. The acid hydrolyzable substances were shown to be intimately associated with the reducing sugars, being in equilibrium with them and fluctuating in the same direction. When the fluctuations in reducing sugars are sufficiently great, not only do the acid hydrolyzable substances respond, but also the residual polysaccharides. The latter are thought to include the remaining portion of the "intussusception" material of the cell wall which is not taken out in the acid hydrolyzable fraction.

Thus it appears that all carbohydrates, with the probable exception of the primary cellulose wall, are actively or potentially functional in the metabolism of the plant. The "exceedingly complex dynamic equilibrium" of which SPOEHR (30) writes extends beyond the hemicelluloses to include the cell wall polysaccharides and their derivatives. The activity of this group in carbohydrate metabolism depends upon: the kind of plant; the amounts of other carbohydrates present; the nature and stability of the polysaccharides and

derivatives making up the hemicelluloses; the residual substances; and, what is still more important, the magnitude of the shift in the "dynamic equilibrium."

Summary

1. The seasonal fluctuations in reducing sugars, sucrose, starch, acid hydrolyzable carbohydrates, total carbohydrates, and the residual polysaccharides are recorded for the raspberry plant.

2. Double rings, one 15 cm. and another 30 cm. above the ground, isolating a 15 cm. segment of stem, were used to check movements of sugars.

3. In normal plants the concentration of total carbohydrates is fairly high in May, drops during June, the period of most active growth, and then increases to a maximum in August. The acid hydrolyzable carbohydrates are the largest fraction of the total carbohydrates. The concentration of reducing sugars is highest in May, drops rapidly in June, and increases slightly in July and August. A low concentration of sucrose is present at all times. Starch is never abundant, and apparently is not an important carbohydrate reserve.

4. In ringed plants the total carbohydrates accumulate above the rings, and decrease below and between them. Sucrose made the largest increase above the ring. Reducing sugars accumulated above the rings, but decreased between and below them. Apparently sucrose tends to move upward as well as downward in the bark. It does not appear to be a product of hydrolysis of hemicellulose in the isolated segment as do reducing sugars. A portion of the reducing sugars is translocatory, moving downward in the bark.

5. All carbohydrate gradients are positive. Sugars move downward with a marked positive gradient. The positive gradients of the acid hydrolyzable substances and of starch are static. These gradients are produced by temporary storage brought about by accumulations within living cells and cells associated with living cells. Ringing decreases the positive gradients of reducing sugars, indicating that some of them are mobile in the bark along a dynamic positive gradient. Much of the reducing sugars may be a temporary storage

form and not functional in translocation. Therefore their positive gradient is the result of a positive dynamic gradient of mobile sugars and a positive static gradient of temporary storage sugars.

6. The insignificant and probably non-existent gradient of sucrose is increased rather than decreased, as would be expected if this sugar were moving only downward.

7. The residual substances consist of those polysaccharides, small quantities of fats, minerals, and organic nitrogen which remain after the total carbohydrates, including acid hydrolyzable substances, are subtracted from the dry weight. The residual polysaccharides are thought to include the "intussusception" material (polysaccharides of high molecular weight and their derivatives) of the cell wall. The residual polysaccharides fluctuate as the total carbohydrates (particularly reducing sugars and the "hemicelluloses") fluctuate, and in the same direction.

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